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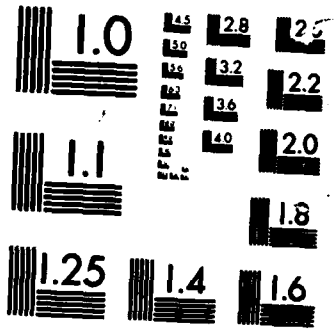

MICROCOPY RESOLUTION TEST CHART

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TRYPANOSOME SURFACE ANTIGEN GENES: ANALYSIS USING RECOMBINANT DNA

FINAL REPORT

12/1/81 - 8/31/84

Kenneth D. Stuart, Ph.D.

March 27, 1985

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701-5012

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Issaquah Health Research Institute
1595 N.W. Gilman Blvd.
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The objective of this project is to identify and isolate early (frequently expressed) variant antigenic types (VATs), and clone and characterize their expressed variant surface glycoprotein (VSG) genes. Numerous syringe passaged and cyclically transmitted, frequently expressed VATs have been isolated, monoclonal antibodies prepared to their VSGs, and the expressed VSG genes have been cloned. We have shown that many diverse stocks express VSG epitopes related to the early IsTat epitopes. The VSG gene organization has been characterized. We have confirmed sequence homology at the 3' terminus of the VSG genes and have discovered additional homology near the 5' terminus of unrelated VSG genes. Numerous relapse VATs have also been isolated and characterized with respect to telomeric location of VSG

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genes and duplicative or non-duplicative mode of VSG gene expression. We have found that two fundamental processes control the expression of VSG genes. One involves gene duplication; the other telomeric activation. In addition, the telomeric location of the gene affects its probability of expression and stability. DNA sequence analysis in and around flanking VSG genes has characterized some of the internal homology and identified flanking sequence homology blocks. The latter probably function in duplicative activation of VSG genes. Key words: African trypanosomes, Sleeping Sickness.

SUMMARY

The purpose was to isolate and characterize early variant antigenic types (VATs) and the corresponding variant surface glycoprotein (VSG) genes of African trypanosomes.

METHODS

The VATs were isolated from chronic and relapse infections by cell cloning. The VSGs were purified from these VATs by affinity chromatography and used to produce VSG specific monoclonal antibodies. These antibodies were used to characterize VAT populations. VSG gene sequences were isolated by molecular cloning of cDNAs which were in turn used as probes to isolate VSG gene sequences cloned from genomic DNA. These cloned VSG gene sequences were characterized directly by restriction mapping and nucleotide sequence analysis and were used as probes to examine the organization and expression of the VSG genes and the process of antigenic variation.

RESULTS AND CONCLUSIONS

The VSG genes expressed by early VATs are located near telomeres of minichromosomes or larger chromosomes indicating that telomeric genes are preferentially expressed. Telomeric genes can be replaced by gene conversion showing that the repertoire is not stable. Several factors including VAT growth rate and switching frequency affect the sequence of VAT occurrence and the VAT composition of relapse populations. The IsTat 1 early VATs occur frequently in several stocks of African trypanosomes indicating that the antigen genes are conserved and expressed in several natural populations.

VSG genes conserve certain sequences in their 5′and 3′ flanks and in their coding sequences. A V-sequence constitutes most of the barren region of telomeric VSG genes and occurs in the 5′ flank of some, but not all, intrachromosomal VSG genes. This sequence occurs near the 5′ boundary of gene conversion and may have a role in this process but it cannot yet be determined if it has a role in controlling VSG gene expression. Most, but not all, VSG genes also conserve a 3′ sequence. All telomeric VSG genes conserve a more extensive sequence and also conserve a CCCTAA containing sequence that may be a characteristic of the telomere. Sequences are also conserved within the coding sequence. The 3′ ends are similar among VSG genes and a sequence is conserved near the 5′ end of the coding sequence. The conserved 3′ end may function in associating the VSG to the cell membrane; the function of the 5′ conserved region is not known.

The VSG genes exist as families of related sequences within chromosomes and near telomeres. The VSG genes are expressed at telomeres. Two processes mediate antigenic variation. One is a gene conversion mechanism that replaces one expressed VSG coding sequence with a copy of another. The second mechanism transcriptionally activates an alternate VSG gene and inactivates the former VSG gene. These processes are independent. In addition, transcripts of VSG genes are processed to remove 5′ sequences; they also have a 35 nucleotide sequence spliced onto their 5′ terminus. The addition of this 35 nucleotide sequence is a novel mechanism and is not restricted to VSG genes.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

AD_____

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(1.) OBJECTIVES

The overall objective of this project was to investigate the characteristics of variant surface antigen (VSG) genes in African trypanosomes in order to determine if they had common characteristics that might be of value for immunoprophylaxis. This integrated biological/immunological/molecular genetic project had the following specific objectives for the period of support:

1.1 To identify and characterize frequently occurring and cyclically transmitted variant antigenic types (VATs). These experiments were designed to identify factors that are important to the frequent occurrence of VATs.

1.2 Examine the VSG genes of frequently occurring VATs to seek common characteristics within these genes to determine if they contain common sequences.

1.3 To characterize the flanking sequences of early and later VAT VSG genes. These experiments are designed to determine the molecular basis for the frequent expression of some VSG genes.

1.4 Determine the occurrence of IsTar 1 VATs among various stocks of African trypanosomes. These studies are designed to determine if particular epitopes are conserved among various stocks of trypanosomes.

(2.) PROGRESS REPORT

Since all of the results that are reported here have been reported on in detail in annual reports starting 12/1/81, 12/1/82, and 12/1/83, and in publications cited at the end of this report, this progress report will summarize the overall progress during the contract period.

(3.) BIOLOGICAL

We have produced a serodeme of antigenic variants from a clone of the EATRO 164 stock. This serodeme contains three general classes of variant antigenic types (VATs): 1. various VATs occurring early or later during the course of a single infection. We have performed most of our studies with six VATs designated A, 1, 3, 5, 7, and 11 which were isolated in this order. 2. Relapses to the above six VATs which resulted from single antigenic switches. These VATs were useful since we knew the identity and characteristics of the VATs before and following an antigenic switch. 3. VATs that were isolated following cyclic transmission and VATs from other stocks that were immunologically cross reactive with the six VATs above.

Several biological parameters of these VATs were determined. These included the relative growth rates and VAT switching frequencies and the order of occurrence of the six VATs in first and subsequent relapses. We found that clones expressed VATs in characteristic orders but that this order could be modified as a

result of genomic rearrangement. These results show that while each serodeme expresses VATs in a characteristic order this order is not fixed but drifts. We also found that growth rate and switch frequencies were characteristic to each VAT and that these were important factors in the sequence of occurrence of VATs during an infection. Our molecular genetic studies suggest that the genomic location and the character of the flanking sequences affect expression frequency but we could not discern a basis for the differences in growth rates among VATs. These data taken together indicate that several factors influence the sequence of VAT occurrence and that the sequence undergoes spontaneous drift during the course of an infection.

(4.) IMMUNOLOGICAL

We have raised both monoclonal and polyclonal antibodies that are specific for the VSGs of the six VATs described above. These antibodies have been used to characterize the populations used for molecular studies and also for the studies of sequence of VAT occurrence. Most of the monoclonal antibodies did not react with the surface of live cells and did not cross react with different VSGs, but all reacted with isolated VSGs, or cell lysates in ELISA assays and with acetone fixed cs by immunofluorescence. Some, but not all, of the monoclonal antibodies reacted with in vitro translation products and with denatured proteins as determined by western blot experiments. These studies suggest that most of the antibodies are directed against polypeptide epitopes that are not exposed on the surface of live cells. Competitive immunofluorescence studies indicated that all monoclonal antibodies reacted with distinct epitopes.

(5.) MOLECULAR GENETIC

Several cDNAs were isolated for each of the six VATs that we studied. Taken together these cDNAs represented the entire coding sequence of most of these VSG genes. Each of these cDNAs has been characterized by restriction mapping and cross hybridization and the nucleotide sequence of many of the cDNAs has been determined. Some of the cDNAs contain a 35 nt sequence on their 5′ terminus. This is the spliced leader (SL) sequence that occurs on the 5′ terminus of all mRNAs in trypanosomes. Several of the cDNAs also have poly (A) tails indicating that they represent the 3′ terminus of the coding sequence. We have found one region of cross hybridization among the cDNAs that is located a few hundred nucleotides from the 5′ terminus but have not identified this region by sequencing. We have found conserved sequences within the 3′ untranslated region of the sequence that has the character of CTCC , AT-rich, and a 123 mer that are conserved in this order. At least one of the cDNA clones expresses the VSG protein in E. coli and these studies showed that codon usage while employing several codons that are rarely used in coli did not prevent expression.

The cDNAs were used as probes to determine the number and organization of the VSG genes in the genome of VATs expressors

and non expressors of the corresponding VSGs. We found that there were 2, 0, 10, 3, 2, and 3 intrachromosomal members of the A, 1, 3, 5, 7, and 11, respectively, VSG gene families. In addition there were varying numbers of telomeric VSG genes depending on the particular clone of the VAT examined. Invariably, the gene that was expressed was located on a telomere. There were 1, 3, and 5 VAT VSG genes located on telomeres of minichromosomes. The 3 and 5 VSG genes served as basic copies for gene conversion leading to expression of the corresponding VAT. The minichromosomal 1 VSG gene was expressed without duplication on a minichromosome. The telomeric A VSG gene was located on an approximately 2000 kb chromosome. This gene could be expressed directly by telomeric activation or serve as a basic copy for the generation of another telomeric VSG gene. In addition the telomeric A gene could be lost leaving no telomeric A VSG genes.

The cDNAs were used as probes to isolate genomic clones that contained intrachromosomal VSG genes from lambda 1059 genomic libraries. They were also used as probes to clone the telomeric 1 VSG gene that was isolated from genomic DNA that had been treated with Bal 31 and a restriction enzyme. These genomic clones have been mapped in detail and the coding sequences have been located. Several segments of these genomic clones have been subcloned and used as probes and sequenced. These studies showed that many, but not all, intrachromosomal genes contained related, but not identical, sequences in their 5′ and 3′ flanks. The 5′ flank contained a 76 nt sequence that occurred as a single sequence or as tandem repeats. This sequence often, but not always, contained a long sequence of TAA in tandem up to 109 times. The rest of the sequence had an alternating GT then alternating TA character. This sequence has the potential to be a B to Z transition region. This sequence occurred approximately at the 5′ boundary of gene conversion and thus may be a recognition sequence for this event. The variation among these sequences may be a factor that contributes to the difference in switching frequencies among some VATs. The 3′ flanking sequence also contained conserved sequences that varied among VATs. They were less extensive in size and less variable and had the CTCC, AT-rich then conserved 13 nt character. These sequences may represent the 3′ terminus of gene conversion. The VSG genes are clustered in tandem arrays in the genome but members of the same family are not in tandem.

The analyses of transcription have shown that VSG genes are transcribed as a larger precursor. However, the coding sequence contains no introns and all processing removes 5′ sequences. However, all VSG (and other) mRNAs contain a 35 nt SL sequence on the 5′ terminus that is transcribed from a large tandem array of a 1.4 kb sequence. The SL transcript is about 140 nt with the 35 nt sequence at its 5′ terminus. The sequence from which the SL is transcribed is not cis to all VSG genes but the SL becomes spliced to the 5′ end to the mRNA. The mechanism by which this occurs is unknown.

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